REMARKS

Claims 1, 2, 4-10, 13, 15, 16, and 18 are pending in the instant application. The claims as pending are attached hereto as *Appendix B*.

I. The Amendments

Substitute Specification. Applicants submit herewith a Substitute Specification, as *Appendix D*, and a marked up copy of the Substitute Specification in accordance with 37 C.F.R. § 1.125, as *Appendix C*. The Substitute Specification is prepared solely for the purpose of complying with the rules of practice; it does not introduce new matter. The marked up copy of the Substitute Specification compares the Substitute Specification with the certified English translation of German priority document no. 197 31 741.3 (previously submitted) to show matter added and deleted from the original Specification. It is submitted herewith in compliance with 37 C.F.R. § 1.125(b)(2).

Applicants request entry of the Substitute Specification and the marked-up copy of the Substitute Specification into the instant application.

In the Claims. Claims 1, 5, 7, 8, 9, 10, 13, 15, and 16 have been amended and new Claim 18 has been added, without prejudice, for the purpose of correcting typographical errors and of more clearly defining what Applicants regard as the invention. Applicants reserve the right to pursue canceled or amended subject matter in one or more timely filed continuation or continuation-in-part applications. The amendments do not introduce new matter and they are fully supported by the Specification of the present application and the

claims as originally filed, as specified below. Therefore, entry of the amendment under 37 C.F.R. § 1.111 is respectfully requested.

More specifically, Claim 1 has been amended to correct a typographical error introduced in the Preliminary amendment which caused the term "acidic amide bond" to be inadvertently changed to "acidic amine bond." Further, the term "enane bridge" is amended to properly read "Schiff base." Claims 4 and 9 have been rewritten to read in independent form. Claims 1, 5, 8, 9, 10, 13, 15, 16 have been amended to clarify some terms objected to by the Examiner. New Claim 18 has been added to claim separately some of the aspects previously claimed by Claim 16.

A marked-up copy of the amended claims is attached hereto as *Appendix A*. The claims as pending after entry of the instant amendments are attached hereto as *Appendix B*.

II. Species Election

Applicants' species election is objected to because, according to the Examiner, Claim 1 is not reading on the elected species "sulfonamide linkage" as the nature of bond between carrier and fluorescent moiety. Applicants submit that the examiner's concern is addressed by correcting a typographical error in Claim 1, which now recites "acidic amide bond," which encompasses the elected species. Therefore, the objection should be withdrawn.

III. Rejections

A. Rejection Of Claims 1, 2, 4-10, 13, 15, And 16 Under 35 U.S.C. § 112, First Paragraph For Lack Of Written Description

Claims 1, 2, 4-10, 13, 15, and 16 are rejected under 35 U.S.C. § 112, first paragraph for allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors had possession of the claimed invention at the time of application. The rejections are obviated and/or overcome by the amendments to Claims 1 and 13.

First, the Examiner asserts that the specification contains no support for the terms "acidic amine bond" and "enane bridge" recited in Claim 1. The term "acidic amine bond" was inadvertently introduced in the Preliminary Amendments filed January 21, 2000. The term in the original Claim 1 was "acidic amide bond" which, as the Examiner points out, is adequately disclosed on page 4, line 5. The amendment of Claim 1, submitted herein, corrects this error, thus making the Examiner's rejection with regard to this term moot.

With regard to the term "enane bridge," Applicants submit that this term represents an inaccurate translation of the German term "Enan Brücke," which, as stated in the specification, is the same as a Schiff base. *See*, Substitute Specification at page 2, line 12 (*see* also German application at page 1, line 19). For purposes of clarity, Applicants amended Claim 1 to recite a "Schiff base" in place of an "enane bridge." This amendment moots the rejection with regard to the term "enane bridge."

Thus, in view of the above, the rejection of Claim 1, and the claims that depend therefrom, under 35 U.S.C. 112, first paragraph, for lack of written description, should be withdrawn.

With regard to Claim 13, the Examiner asserts that the phrase "comprising a serum albumin" in Claim 13 is not supported by the specification. This rejection is obviated and/or overcome by the amendment to Claim 13.

Accordingly, Applicants respectfully request that the rejection of Claim 13, and the claims that depend therefrom, under 35 U.S.C. 112, first paragraph, for lack of written description should be withdrawn.

B. Rejections Of Claims 1, 2, 4-10, 13, 15, And 16 Under 35 U.S.C. § 112, First Paragraph For Lack Of Enablement

Claims 1, 2, 4-10, 13, 15, and 16 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to make and/or use the claimed invention. The rejection is respectfully traversed.

Specifically, the Examiner asserts that there is no evidence in the specification that the claimed conjugates indeed accumulate in unhealthy tissue, *e.g.*, cancerous or inflamed tissue, as recited in the claims. Applicants are in the process to prepare and will submit a Declaration under 37 C.F.R. § 1.132 by Dr. Sinn substantiating enablement of the presently claimed application, showing experimental data confirming accumulation of the claimed conjugate in a cancerous tissue and inflamed tissue, respectively.

Claim 16 has been amended in accordance with the Examiner's suggestion to eliminate the term "pharmaceutical," making rejection with regard to that term moot. In view of the above, Applicants respectfully request that the rejection of Claims 1, 2, 4-10, 15, and 16 under 35 U.S.C. § 112, first paragraph, for lack of enablement, be withdrawn.

C. Rejection Of Claims 1, 2, 4-10, 13, 15, And 16 Under 35 U.S.C. § 112, Second Paragraph

Claims 1, 2, 4-10, 13, 15, and 16 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. This rejection is in part traversed and in part obviated and/or overcome in view of the amendments to the claims.

First, the Examiner objects to the terms "acidic amine bond," "enane bridge," and "acidic ester." With respect to the terms "acidic amine bond" and "enane bridge," the rejection is moot in light of the amendment to Claim 1. An "acidic amide bond," as recited, is exemplified by the sulfonamide linkage between the carrier and the fluorescent moiety of the conjugate shown in FIGURE 3. Additionally, Applicants disclose several specific examples of fluorescent compounds and carrier molecules, as well as the relevant groups included on those compounds, which could be used to practice the invention. Such examples may be found in the Specification, for example, at page 2, lines 17-19 and 24-26 (disclosing examples of carrier molecules), and page 3, lines 10-13 and 18-26 (disclosing examples of fluorescent compounds). In light of these representative molecules, one skilled in the art would be able to design appropriate reactions to generate any of the claimed bond types found in Claim 1. Accordingly, Applicants respectfully request that the instant rejection of Claim 1 be withdrawn.

Second, the Examiner asserts that recitation of a conjugate comprising a "compound" is indefinite since a conjugate is a single compound. This rejection is obviated and/or overcome in view of the amendment to the claims. Specifically, in accordance with the Examiner's suggestion, Applicants have amended Claim 1 and the claims that depend

therefrom to recite a "fluorescent moiety" rather than a "fluorescent compound." Accordingly, Applicants respectfully request that the instant rejection of Claim be withdrawn.

Third, the Examiner contends that the term "joined" as used in Claim 1 is an inexact term. Applicants respectfully traverse. The term "conjugate" is well recognized in the art as relating to two molecules being joined, either covalently or noncovalently. This definition is further supported by the dictionary definition of "conjugation" which recites "the covalent or noncovalent joining together" of two molecules. *Oxford Dictionary of Biochemistry and Molecular Biology, Revised Edition*, Smith, A.D. et al., Eds. (2000) at 134. Therefore, use of the term "joined" is not inexact. However, in order to reduce the issues, the Applicants amended Claim 1 in accordance with the Examiner's suggestion. Accordingly, the rejection of Claim 1 over the term "joined" is moot and should be withdrawn.

Fourth, Claim 8 is found indefinite for the recitation of the term "acridic acid," because this term is not found in the Chem. Abstracts database. Applicants submit that the term is a proper translation of the German term "Akridinsäure," as evidenced by Gerhard Wenske, Dictionary of Chemistry - English/German, VCH, Weinheim, New York, Basel, Cambridge, at page 13. *See, Exhibit 1.* An "acridic acid" is an acid of acridine. Accordingly, the rejection of Claim 8 over the term "acridic acid" is in error and should be withdrawn.

Fifth, Claims 4 and 9 are found indefinite as being broader than Claim 1. This rejection is obviated and/or overcome by the amendment to Claims 4 and 9, which are now written independent form. Accordingly, the rejection should be withdrawn.

Sixth, Claim 8 is found to be rendered indefinite by the term "derivative." This rejection is obviated and/or overcome by the amendment to Claim 8 and should therefore be withdrawn.

Seventh, Claim 10 is found to be rendered indefinite by the phrase "covelently bonded." This rejection is obviated and/or overcome by the amendment to Claim 10.

Finally, Claim 15 is found to be unclear because of the recitation of ranges of different wavelengths. This amendment is obviated and/or overcome by the amendment to Claim 15 and the addition of new Claim 18, as proposed by the Examiner.

In view of the above, the rejection of Claims 1, 2, 4-10, 13, 15, and 16 under 35 U.S.C. § 112, second paragraph, should be withdrawn.

IV. Information Disclosure Statement

Applicants submit herewith a copy of U.S. Patent No. 4,659,657, *see*, *Exhibit 2*, which is based on DE 32 48 043 (PTO-1449 form reference AF) and DE 33 21 041 (PTO-1449 reference AE), and a copy of U.S. Patent No. 6,120,987, *see, Exhibit 3*, which is based on FR 2,757,162 (PTO-1449 reference AG). Applicants respectfully request that these references be considered by the Examiner.

CONCLUSION

In view of the above amendments and remarks, the subject application is believed to be in good and proper order for allowance. Early notification to this effect is earnestly solicited.

If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned at (650) 493-4935.

The commissioner is authorized to charge any underpayment or credit any overpayment to Deposit Account No. 16-1150 for any matter in connection with this response, including any fee for extension of time, which may be required.

Respectfully submitted,

Date 7/3/02

47,763

Nathan A. Machin (Reg. No.)

For: Laura A. Coruzzi (Reg. No. 30,742)

PENNIE & EDMONDS LLP
1155 Avenue of the Americas
New York, New York 10036-2711
(650) 493-4935

Enclosures

APPENDIX A
Serial No.: 09/463,474
Marked-up Copy of the Claims

1. (Thrice Amended) A conjugate for distinguishing [unhealthy] tissue from healthy tissue comprising a fluorescent [compound] moiety and a carrier, wherein the fluorescent [compound] moiety and the carrier are joined to one another via an acidic ester, an acidic [amine] amide bond or [an enane bridge] a Schiff base, and wherein said carrier is a protein.

2. (Reiterated) The conjugate of claim 13, wherein the serum albumin comprises a human serum albumin.

4. (Twice amended) [The conjugate of Claim 1] A conjugate for distinguishing cancerous or inflamed tissue from healthy tissue comprising a fluorescent moiety and a carrier, wherein the fluorescent moiety and the carrier are joined to one another via an acidic ester, an acidic amide bond or a Schiff base, wherein said carrier is a protein, and wherein the conjugate comprises a plurality of carriers.

5. (Twice Amended) The conjugate of claim 1, wherein the fluorescent [compound] moiety comprises an acid group, a hydroxyl group, an amino group or an aldehyde group.

6. (Reiterated) The conjugate of claim 15, wherein the excitation wavelength is 630 to 850 nm.

7. (Twice Amended) The conjugate of claim [15] 18, wherein the excitation wavelength is 320 to 450 nm.

8. (Twice Amended) The conjugate of claim 1, wherein the fluorescent [compound] moiety comprises a porphyrin, [a porphyrin derivative,] a chlorin, [a chlorin derivative,] a bacteriochlorin, [a bacteriochlorin derivative,] a chlorophyll, [a chlorophyll derivative,] a phthalocyanine, [a pthalocyanine derivative,] a carboxy cinnamic acid, a carboxy cinnamic acid, a carboxyfluorescein, [a carboxyfluorescein derivative,] an acridic acid, [an acridic acid derivative,] a coumaric acid, [a coumaric acid derivative,] or an indocyanine green [or an indocyanine green derivative].

9. (Twice Amended) [The conjugate of claim 1] A conjugate for distinguishing cancerous or inflamed tissue from healthy tissue comprising a fluorescent moiety and a carrier, wherein the fluorescent moiety and the carrier are joined to one another via an acidic ester, an acidic amide bond or a Schiff base, and wherein said carrier is a protein, and wherein the conjugate comprises a plurality of fluorescent [compounds] moieties.

10. (Twice Amended) A method of producing the conjugate of claim 1, wherein the fluorescent [compound] moiety [and] is covalently bonded to the carrier [are covalently bonded] thereby forming the connector.

13. (Amended) The conjugate of claim [12] 1, wherein the protein [comprises] is a serum albumin.

15. (Amended) The conjugate of claim 1, wherein the fluorescent [compound] moiety has an excitation wavelength of 630 nm or greater [or 450 nm or less].

16. (Amended) A [pharmaceutical] composition comprising the conjugate of claim 1 and an acceptable carrier or excipient.

Please add new Claim 18:

18. (New) The conjugate of claim 1, wherein the fluorescent moiety has an excitation wavelength of 450 nm or less.

APPENDIX C
Serial No.: 09/463,474
Marked-Up Copy Of The Substitute Specification

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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

CONJUGATE FOR DIFFERENTIATING BETWEEN HEALTHY AND UNHEALTHY TISSUE

This is a national phase filing of the Application No. PCT/DE98/02102, which was filed with the Patent Corporation Treaty on July 22, 1998, and is entitled to priority of German Patent Application 197 31 741.3 filed July 23, 1997.

I. FIELD OF THE INVENTION

The present invention relates to conjugates for differentiating between healthy and unhealthy tissue, methods of producing such conjugates as well as their use.

II. BACKGROUND OF THE INVENTION

For the treatment of unhealthy tissue, *e.g.* of tumors, the removal thereof is often an essential measure. For this purpose, it is necessary for the operating surgeon to recognize accurately where unhealthy tissue ends and where healthy tissue starts. However, this is often impossible. As a result, offshoots of the unhealthy tissue are overlooked, which are then the basis for another formation of the unhealthy tissue.

Therefore, it is the object of the present invention to provide a product by means of which a differentiation can be made between unhealthy and healthy tissue.

III. SUMMARY OF THE INVENTION

The present invention relates to conjugates for differentiating between healthy and unhealthy tissue, methods of producing such conjugates as well as their use.

IV. BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows the production of a conjugate from acridine-9-carboxylic acid and human serum albumin.

FIGURE 2 shows the production of a conjugate from coumarin 343 and human serum albumin.

FIGURE 3 shows the production of a conjugate from tetrasulfophenylporphin and human serum albumin.

V. DETAILED DESCRIPTION OF THE INVENTION

It is the object of the present invention to provide a product by means of which a differentiation can be made between unhealthy and healthy tissue. According to the invention this is achieved by the subject matters defined in the claims.

Thus, the subject matter of the present invention relates to a conjugate, comprising a fluorescent compound and a carrier, wherein the compound and the carrier are connected via an acidic ester or acidic amide bond or enane bridge (schiff base) and the compound has an excitation wavelength of 630 nm or more and/or 450 nm or less.

The expression "carrier" comprises compounds of any kind which are suited for the enrichment of the conjugate in a certain tissue, *e.g.* a tumor, a focus of inflammation or in superficial, relatively small vessels, such as neovascularizations in the area of the cornea. Examples of such carriers are proteins and polyether. For forming the acidic ester or acidic amide bond with the fluorescent compound, the carrier may include hydroxyl or amino groups.

The proteins are preferably not considered foreign to the body. They may be present in native form. In the native form, the proteins have no intermolecular and/or intramolecular cross-linking. The proteins favorably have a molecular weight of up to 100,000 Dalton, particularly 30,000 to 100,000 Dalton. Furthermore, it is favorable for the proteins to be human proteins. Examples of the proteins are albumin, fibrinogen, transferrin, immunoglobulins and lipoproteins, human serum albumin (HSA) being preferred. It is also possible to use fragments of the above proteins. In addition, the sequence of the proteins and the fragments thereof, respectively, may comprise modifications of one or several amino acids over known sequences of the proteins and fragments thereof, respectively.

Examples of the polyethers are polyethylene glycols, particularly those having a molecular weight of 100 to 20,000 Dalton. The polyethylene glycols are preferably esterified or etherified with a C_1-C_{12} alkyl group, particularly with a methyl group, on the terminal hydroxyl group.

A conjugate according to the invention may have one or several, particularly 2 to 4, of the above carriers. [i]If several carriers are present, they may be equal or differ from one another. If several polyethers are present, they will favorably be selected such that the molecular weight of all polyethers is about 20,000 Dalton or more.

The expression "fluorescent compound" comprises compounds of any kind which can be induced to display fluorescence. These compounds can also be photoactive. The compound is connected with the carrier via an acidic ester or acidic amide bond or enane bridge. For the formation thereof, the fluorescent compound may comprise an acid group, *e.g.* a carboxylic, sulfonic, phosphonic or arsonic acid group, a hydroxyl group, an amino group or an aldehyde group. Several of these groups may be present, which may be equal or differ from one another. The fluorescent compound is excited at a wavelength of 640 nm or more, preferably 630 to 850 nm, and particularly preferably 650 to 850 nm, and/or at a wavelength of 450 nm or less, preferably 320 to 450 nm. These wavelengths refer to the excitation wavelengths which the fluorescent compound has in the conjugate according to the invention; in a free form, their excitation wavelength may differ therefrom. Representatives of these compounds are porphyrins such as tetrasulfophenyl porphyrin (TSPP; excitation wavelength 650 nm when bound to HSA), chlorins, bacteriochlorins, chlorophylls, phthalocyanines, wherein these compounds may include metal ions as central atom. Furthermore, representatives of the fluorescent compound are carboxy cinnamic acid, carboxy fluorescein, acridine carboxylic acid, such as acridine-9-carboxylic acid, coumaric acid, such as coumarin 343, coumarin-3-carboxylic acid, and hydroxy coumarin acetic acid (excitation wavelength 365 nm when bound to HSA), and indocyanine green (excitation wavelength 805 nm when bound to HSA) as well as derivatives of the above compounds.

One or several fluorescent compounds can be present in the conjugate according to the invention. If several are present, they may be the same or differ from

one another. Particularly preferred conjugates according to the invention are shown in [figures] FIGURES 1 to 3.

Conjugates according to the invention can be produced by covalently bonding the fluorescent compound with the carrier thereby forming an acidic ester or acidic amide bond. A person skilled in the art is familiar with methods suitable for this purpose as well as necessary materials.

If the fluorescent compound includes an acid group, the conjugates can be produced by reacting this compound with carbodiimide and hydroxy succinimide into reactive succinimidyl esters and the latter can then be converted with the carrier. In the case of conjugates having several fluorescent compounds, the succinimidyl esters can be produced jointly or separately.

The fluorescent compound is reacted with carbodiimide and hydroxy succinimide in a polar aprotic solvent, preferably dimethyl formamide or dimethyl sulfoxide (DMSO). The molar ratio of fluorescent compound : carbodiimide : hydroxy succinimide is about 1 : 1.5-3 : 5-10. The resulting succinimidyl ester is then reacted in an aqueous buffer solution, preferably $NaHCO_3$, with the carrier, such as albumin. The carrier concentration is about 10 to 70 mg/ml. The thus activated acid group can then react with OH and NH groups of the carrier thereby forming acidic amide or acidic ester bonds, conjugates according to the invention being obtained. The conjugates can be purified several times, *e.g.* by ultrafiltration, and finally be sterile filtered. Thereafter, they are ready for application.

Conjugates according to the invention distinguish themselves by a prolonged half life in the organism. In addition, conjugates according to the invention accumulate in unhealthy tissue, particularly in tumoral tissue, in foci of inflammation and in superficial relatively small vessels, *e.g.* of neovascularizations in the area of the cornea. The fluorescent compound is excited or activated by light, so that unhealthy tissue can be made visible, whereas healthy tissue in which the conjugates according to the invention to not accumulate is not made visible. Furthermore, there is no disturbance caused by the inherent fluorescence of blood or tissue, *e.g.* the liver, so that the optical impression is not falsified. In addition, conjugates according to the invention, in which

the fluorescent compound can be excited at 630 nm or more, have a great penetration depth.

[Brief description of the drawings:

Figure 1: shows the production of a conjugate from acridine-9-carboxylic acid and human serum albumin,

Figure 2: shows the production of a conjugate from coumarin 343 and human serum albumin, and

Figure 3: shows the production of a conjugate from tetrasulfophenylporphin and human serum albumin.]

The [following] below examples explain the invention[:] in more detail. The following preparations and examples are given to enable those skilled in the art to more clearly understand and to practice the present invention. The present invention, however, is not limited in scope by the exemplified embodiments, which are intended as illustrations of single aspects of the invention only, and methods which are functionally equivalent are within the scope of the invention. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

VI. EXAMPLES

A. Example 1: Production of a Conjugate According to the Invention from Acridine-9-carboxylic Acid and Human Serum Albumin

The structure and the production of the conjugate are shown in [figure] FIGURE 1.

20 mg of acridine-9-carboxylic acid hydrate (A9CA) were dissolved in 2 ml DMSO and about 100 mg of N-hydroxysuccinimide (HSI) in a molar ratio of about

10/1 as well as 30 mg N,N'-di-cyclohexyl carbodiimide (DCC) in a molar ratio or about 1.5/a were added. After about 6 hours, the formation of the hydroxysuccinimidyl ester is concluded. Following the separation of the dicyclohexyl urea (DCHU) through a solvent-resistant filter (0.2 μm), the ester is slowly added to a solution of 2 g of human serum albumin (HSA) which is dissolved in 10 ml of original solution, 10 ml of 0.34 M $NaHCO_3$ and 10 ml of methoxypolyethylene glycol (MPEG). The slight clouding resulting upon the addition disappears again after a short time. A slightly yellowish solution of a conjugate from A9CA and HSA results. The accompanying substances undesired in the finished preparation, such as excess DCC, HSI, unbound A9CA, DMSO and MPEG, are separated by means of ultrafiltration (exclusion limit 10 kD) comprising at least 4 wash steps.

B. Example 2: Production of a Conjugate According to the Invention from Coumarin 343 and Human Serum Albumin

The structure and the production of the conjugate are shown in [figure] FIGURE 2.

20 mg of coumarin 343 (C343 = 10-carboxy-2,3,6,7-tetrahydro-1H,5H,11H-[1]benzopyranone[6,7,8,ij]-quinolizine-11-one) were dissolved in 2 ml DMSO. For this purpose, about 100 mg HSI in a molar ratio of 10/1 and 30 mg DCC in a molar ratio of about 1.5/1 were added. The ester was isolated as described in Example 1 and reacted with HSA, an intensely yellow solution of a conjugate from C343 and HSA being obtained. Undesired accompanying substances are separated as described in Example 1.

C. Example 3: Production of a Conjugate According to the Invention from Tetra-(4-sulfophenyl)porphin and Human Serum Albumin

The structure of the conjugate and its production are shown in [figure] FIGURE 3.

Tetra-(4-sulfophenyl)porphin (TSPP) was dissolved in a concentration of 10 mg/ml in DMSO. Three times the molar amount of DCC and five times the molar amount of HSI were added to the clear dark green solution. After a reaction period of about 3 to 4 hours, the conversion into TSPP succinimidyl ester (TSPP-SE) is

concluded, the resulting di-cyclohexyl urea being separated in the form of fine grains. The analytical control is carried out by means of thin-layer chromatography.

Human serum albumin (HSA, 4 g, *i.e.* 2 ampoules of 2 g in 10 ml each) were diluted with 2 x 10 ml of 0.17 M $NaHCO_3$ and 20 ml of methoxypolyethylene $glycol_{350}$ and charged to a 100 ml Erlenmeyer flask. The above TSPP-SE solution in DMSO was slowly added to this HSA solution with constant stirring, the initially clear solution becoming cloudy because of non-reacted DCC which is insoluble in aqueous solution. Having concluded the addition of TSPP-SE, the reaction mixture was stirred at room temperature for 30 minutes so as to complete the reaction. Thereafter, the turbid matter was separated via a sterile filter unit (Millipore, Stericup - GV, 0.22 μm Low Binding Duropore Membrane) and the low-molecular water-soluble components (DMSO, HSI and unbound TSPP) were separated by ultrafiltration via a membrane having 30 kD exclusion limit (Amicon YM 30). A conjugate according to the invention was obtained from TSPP and HSA. The linkage yield of TSPP to HSA was 85 to 90%.

The analytical purity was controlled by means of HPLC under the following conditions:

Precolumn:	Zorbax Diol (50 x 4 mm)
Column 1:	Zorbax GF 450
Column 2:	Zorbax GF 450
Running agent:	0.2 M Na citrate, pH 7.5
Flow:	1 ml/min
Detector 1:	280 nm (for the protein)
Detector 2:	420 m (for TSPP)

All references cited within the body of the instant specification are hereby incorporated by reference in their entirety.

[Claims] **CLAIMS**

WHAT IS CLAIMED:

1. A conjugate, comprising a fluorescent compound and a carrier, wherein the compound and the carrier are connected via an acidic ester or acidic amide bond or an enane bridge, the compound in the conjugate has an excitation wavelength of 630 nm or more and/or 450 nm or less.

2. The conjugate according to claim 1, characterized in that the carrier is a protein.

3. The conjugate according to claim 2, characterized in that the protein is a native protein which is not regarded as foreign to the body.

4. The conjugate according to claims 3, characterized in that protein is human serum albumin.

5. The conjugate according to claim 1, characterized in that the carrier is a polyether.

6. The conjugate according to claim 5, characterized in that the polyether is a polyethylene glycol.

7. The conjugate according to any one of claims 1 to 6, characterized in that several carriers are present.

8. The conjugate according to any one of claims 1 to 7, characterized in that the fluorescent compound comprises an acid group, hydroxyl group, amino group or aldehyde group.

9. The conjugate according to any one of claims 1 to 8, characterized in that the excitation wavelength is 630 to 850 nm.

10. The conjugate according to any one of claims 1 to 9, characterized in that the excitation wavelength is 320 to 450 nm.

11. The conjugate according to any one of claims 1 to 10, characterized in that the fluorescent compound is derived from porphyrin, chlorin, bacteriochlorin, chlorophyll, phthalocyanine, carboxy cinnamic acid, carboxyfluorescein, acridic acid, coumaric acid or indocyanine green as well as the derivatives thereof.

12. The conjugate according to any one of claims 1 to 11, characterized in that several fluorescent compounds are present.

13. A method of producing a conjugate according to any one of claims 1 to 12, characterized in that the fluorescent compound and the carrier are covalently bonded thereby forming an acidic ester or acidic amide bond.

14. Use of a conjugate according to any one of claims 1 to 12 for differentiating between healthy and unhealthy tissue.

[Abstract of the Disclosure]

ABSTRACT

The invention relates to conjugates, comprising a fluorescent compound and a carrier, wherein the compound and the carrier are connected via an acidic ester or an acidic amide bond and the compound has an excitation wavelength of 630 nm or more and/or 450 nm or less. The invention also relates to the production of said conjugates and to the use thereof.

Gerhard Wenske

parat

Dictionary of Chemistry

English/German

Wörterbuch Chemie

Englisch/Deutsch

ISSN 0930-6862

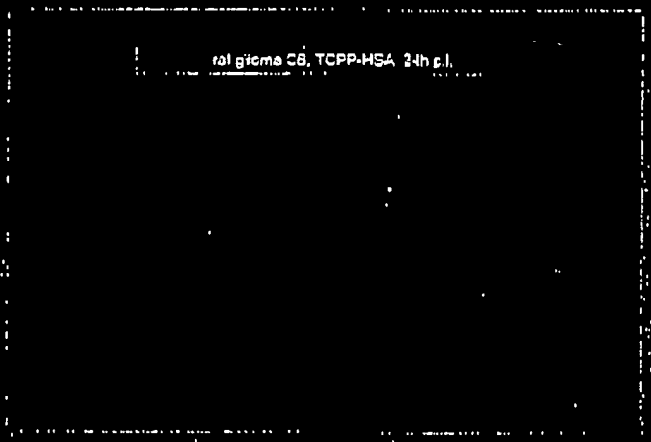
Weinheim · New York · Basel · Cambridge

VCH

acidulated water Sauerwasser *n*, angesäuertes Wasser *n*
acidulating Säuern *n*
acidulation Ansäuern *n*, Ansäuerung *f*, Absäuern *n*, Acidifizierung *f*, Säuerung *f*, Versäuerung *f*
acidulous säuerlich, angesäuert
acierage bath Stählungsbad *n*, Verstählungsbad *n (Beschichtung)*
acierate/to verstählen
acierating Verstählen *n*
acieration Verstählung *f*, Verstählen *n (Beschichten mit Stahl)*
acknowledge/to quittieren, bestätigen; rückmelden *(EDV)*
acmite Akmit *m*, Achmit *m*, Aegirin *m*, Aegirit *m (Min)*
acocantherin Acocantherin *n*
acofriose Acofriose *f*
acolytine Acolytin *n*, Lycacotin *n*
aconic acid Aconsäure *f*
aconitase Aconitase *f (EC 4.2.1.3)*
aconitate Aconitat *n*
aconite Akonit *n (Aconitum napellus)*, Eisenhut *m*, Eisenhutkraut *n*, Sturmhut *m (Bot)*, **aconite root** Akonitknolle *f*, Akonitwurzel *f*, Eisenhutknolle *f (Bot)*
aconitic acid <$HOOCCH_2CHCOOH=CHCOOH$> Aconitsäure *f*, Akonitsäure *f*, Propen-1,2,3-tricarbonsäure *f (IUPAC)*, Achilleasäure *f*, Citridinsäure *f*, Equisetsäure *f*, β-Carboxyglutakonsäure *f*
aconitine <$C_{34}H_{47}NO_{11}$> Aconitin *n*, Akonitin *n (Alkaloid im Sturmhut)*
acorn oil Eichenkernöl *n*
acorn sugar Quercit *m*
acoustic 1. schallschluckend; 2. akustisch
acoustic insulator Schallisoliermittel *n*
acoustic irradiation Beschallung *f*
acoustic paint schalldämpfende Anstrichfarbe *f*, Antidröhnlack *m*, Schallschlücklack *m*
acoustic pressure Schalldruck *m*
acoustic pressure level Schalldruckpegel *m*
acoustic signal Hörzeichen *n*
acoustic wave Schallwelle *f*
acquire/to aneignen, erwerben
acquisition Erfassung *f*
acquisition of knowledge Erfassung *f* von Kenntnissen *fpl*, Wissenserwerb *m*, Wissensakquisition *f*
acquisition of measured data Meßwerterfassung *f*
acremite Acremit *n (obs)*, Ammonsalpeter-Kohlenwasserstoff-Gemisch *n (Expl; 94% NH_4NO_3 16% Heizöl)*
acrid beißend, stechend, ätzend *(Geruch)*; sauer, herb, streng, scharf *(Geschmack)*
acridic acid Acridinsäure *f*, Akridinsäure *f (obs)*
acridine <$C_{13}H_9N$> Acridin *n*, Akridin *n (obs)*
acridine color Akridinfarbstoff *m (obs)*, Acridinfarbstoff *m*
acridine dye Akridinfarbstoff *m (obs)*, Acridinfarbstoff *m*
acridine orange Acridinorange *n*, Tetramethyl-3,6-diaminoacridin-Monohydrochlorid *n*
acridine yellow Acridingelb *n*, Diaminodimethylacridin-Hydrochlorid *n*
acridinium chloride Acriflavin *n*, 2,8-Diamino-10-methylacridinchlorid *n*
acridity Herbe *f*, Schärfe *f*, Herbheit *f*
acridone Acridon *n*, Akridon *n (obs)*
acriflavine Acriflavin *n*, Akriflavin *n*, Trypaflavin *n*, 2,8-Diamino-10-methylacridinchlorid *n*
acrifoline Acrifolin *n*
acritol Acrit *m*
acrolactic acid Glucinsäure *f*, 3-Hydroxypropensäure *f*
acrolein <$CHOCH=CH_2$> Acrolein *n*, Akrolein *n (obs)*, Acrylaldehyd *m*, Allylaldhyd *m*, Propenal *n (IUPAC)*
acrolein resin Acroleinharz *n*
acrometer Öldichtemesser *m*, Ölwaage *f*
acrose Acrose *f*
acryl glass Acrylglas *n*
acrylaldehyde <$CHOCH=CH_2$> Acrylaldehyd *m*, Acrolein *n*, Akrolein *n (obs)*, Allylaldehyd *m*, Propenal *n (IUPAC)*
acrylamide <$CH_2=CHCONH_2$> Acrylamid *n*, Akrylamid *n (obs)*, Propenamid *n*, Acrylsäureamid *n*
acrylamide-gel electrophoresis Acrylamid-Gel-Elektrophorese *f*
acrylate Acrylat *n*, Acrylester *m*
acrylate-acrylonitrile copolymer Acrylat-Acrylnitril-Mischpolymerisat *n*, ANM
acrylate-butadiene rubber Acrylat-Butadien-Kautschuk *m*, Acrylat-Butadien-Gummi *m*, Acrylkautschuk *m*, ABR
acrylate resin Acrylharz *n*
acrylation Einführung *f* eines Säureradikals
acrylhydroxamic acid Acrylhydroxamsäure *f*
acrylic acid <$CH_2=CHCO_2H$> Acrylsäure *f*, Akrylsäure *f (obs)*, Propensäure *f (IUPAC)*, Vinylcarbonsäure *f*, Ethencarbonsäure *f*
acrylic-acid ester copolymer Acrylsäureester-Mischpolymerisat *n*, Acrylsäureester-Copolymerisat *n*
acrylic-acid styrene acrylonitrile copolymer Acrylsäure-Styrol-Acrylnitril-Mischpolymerisat *n*, ASA
acrylic adhesive Acrylkleber *m*, Acrylklebstoff *m*, Acrylklebemittel *n*
acrylic aldehyde <$CHOCH=CH_2$> Propenal *n (IUPAC)*, Acrolein *n*, Acrylaldehyd *m*, Allylaldehyd *m*
acrylic copolymer Acrylmischpolymer *n*

Laserinduzierte Tumordiagnostik und -Therapie

Glioma C6, TCPP-HSA, HE

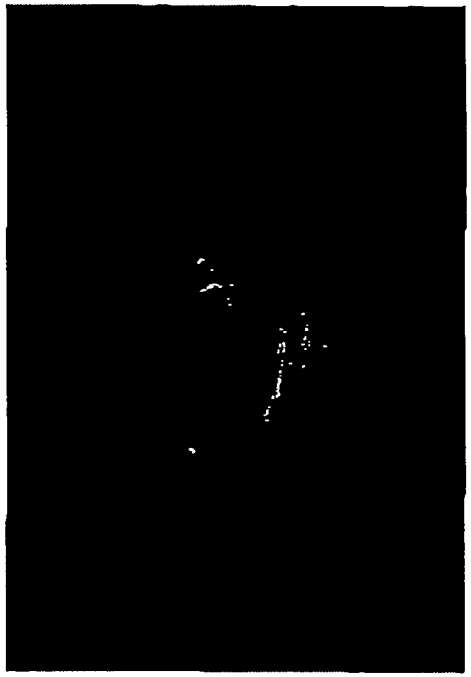


Glioma C6, TCPP-HSA, Fluoreszenz

Lymphknotendarstellung

(mit TCPP/TCPC - HSA)

Weißlicht



UV-Licht